Title:	**Analysis of Bacterial Diversity in Acidic Pond Water and Compost After Treatment of Artificial Acid Mine Drainage for Metal Removal**		**June 2005**
Author(s):	Morales, T.A., M. Dopson, R. Athar, and R.B. Herbert Jr.	**Information Source:**	Biotechnology and Bioengineering, Vol. 90, No. 5, p. 543-551
Technology Type:	Sulfate/Metals Reduction, Bioreactors	**Mine / Facility Type:**	N/A
Study Scope:	Bench-scale/Laboratory	**Mine Name:**	N/A
Source:	Acid mine drainage	**Location:**	Boliden, North Sweden
Contaminant(s):	Metals, Iron, Inorganics, Sulfate	**Receiving Media:**	Ground water, Surface water
Keywords:	sulfate reduction; DGGE; FISH; acid mine drainage; metal remediation		

Abstract: The microbial population of a sludge amended leaf compost material utilized for treatment of artificial acid mine drainage was studied by culture-independent molecular methods. Iron-rich and sulfurous wastewater (artificial acid mine drainage) was circulated through a column bioreactor for 16 months. After 12 months the column was inoculated with a mixed culture from an acidic pond receiving acid mine drainage from a tailings impoundment at a decommissioned site in Kristineberg, North Sweden. Hydrogen sulfide odor and the formation of black precipitates indicated that sulfate-reduction occurred in the column. 16S rDNA gene analysis by denaturing gradient gel electrophoresis, cloning, and sequencing as well as fluorescent in situ hybridization confirmed the presence of microorganisms closely related to sulfate-reducing bacteria and microorganisms from the genera Pseudoxanthmonas, Dechlorosoma, Desulfovibrio, Agrobacterium, Methylocapsa, Rhodococcus, Sulfobacillus, and some unidentified bacteria. Sulfate-reducing bacteria were found in the column bioreactor 2 weeks after inoculation, but not thereafter. This suggests they were in low abundance, even though sulfate remediation rates were significant. Instead, the population contained species similar to those previously found to utilize humic substances released from the compost material.

For more information online:	**Contact:**

Reference

Morales, T.A., M. Dopson, R. Athar, and R.B. Herbert Jr. 2005. Analysis of bacterial diversity in acidic pond water and compost after treatment of artificial acid mine drainage for metal removal. Biotechnology and Bioengineering 90(5): 543-551.

Procedures

Artificial acid mine drainage consisting of iron-rich and sulfurous wastewater was created in a laboratory and pumped through a column containing an organic leaf compost material mixed with cattle/horse manure, sewage sludge, and granite gravel. This mixture circulated at room temperature for one year. Simultaneously, sediment samples were collected from a highly acidic, metal- and sulfur-rich pond adjacent to mine tailings and a wetland at the Kristineberg field site in North Sweden. These samples were cultivated, enriched, and grown in a laboratory for one year. After both mixtures had been circulating for one year, the column containing the artificial acid mine drainage and leaf compost mixture was inoculated with the cultivated environmental sample and lactate. Separate samples were removed after two weeks and two months; after a further year of treatment, the column was spiked with additional lactate and incubated for an additional two weeks before another sample was removed. These samples were analyzed for pH, redox, temperature, ferrous iron, dissolved sulfide, dissolved oxygen, and sulfate. DNA was extracted and PCR was run on each sample. PCR fragments were analyzed using denaturing gradient gel electrophoresis (DGGE) and fluorescent in situ hybridization (FISH) to analyze the microbial population.

Results

The amount of sulfate and iron in the samples decreased over time after innoculation, as did the efficiency of sulfate and iron removal. Sulfate reduction occurred immediately after the cultivated environmental sample was added to the compost, demonstrated by the initial increase in the sulfide concentration. The low average sulfide concentration and high removal of iron in the column suggest that virtually all sulfides were consumed during iron sulfide precipitation. Sulfate reduction is also supported by the black precipitate observed in the column with an iron:sulfur atomic ratio of 0.63.

DGGE showed that different species of microorganisms were found in the samples taken at different time periods. The original environmental sample contained Dechlorosoma, Azospira, and Rhodococcus. After being inoculated with lactate and maintained for a year, Pseudoxanthomonas brogbernesis was also found in the sample. From the environmental sample/leaf compost mixture column sample taken two weeks after innoculation, DGGE identified Dechlorosoma, Desulfovibrio, and Agrobacterium. Dechlorosoma, Rhodococcus, and Sulfobacillus were found in the sample taken two months after inoculation. In the sample taken one year after inoculation, Methylocapsa was the only species found. Finally, in the sample taken 14 months after innocluation, Methylocapsa, Dechlorosoma, Azospira, and Agrobacterium tumefaciens were identified. FISH revealed a diverse community of sulfate-reducing bacteria (SRB) in the cultivated environmental sample, including Desulfovibrio, Desulfotalea, Desulfofusiis, Desulfobacterium, and Desulfomicrobium.

Conclusions

Morales et al. found that the leaf compost mixture successfully removed sulfate from ground and surface waters contaminated with acidic leachate; the initial rate of metal and sulfate removal compared favorably with a previous study. The column mixture in this study was initially demonstrated to contain SRB. After depletion of easily degradable substrate, however, DGGE suggests that potential humic degrading microorganisms dominated over previously found SRB, yet the removal of sulfate and metals continued at a significant rate. The lack of detection of SRB populations after the first two weeks is likely attributable to the fact that DGGE does not detect populations whose abundance is less than 1% (Muyzer et al. 1993), however significant sulfate and metal removal is surprising with SRB abundances less than 1%. Humic-degrading bacteria may either compete with SRB for substrate or work with previously unidentified SRB to link HS degradation to sulfate reduction. Since both molecular techniques utilized in this study (DGGE and FISH) found different microorganism species present in the mixtures during remediation, Morales et al. concluded that species-specific probes and geochemistry data should be used together to control future cost-effective remediation strategies.